

## Gametophyte Ontogeny and Sex Expression in *Dryopteris ludoviciana*<sup>1</sup>

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Although the ontogeny of fern gametophytes has a long history of morphological investigation, sex expression in ferns has only recently been studied by experimental morphologists and geneticists. Most homosporous ferns have bisexual gametophytes whose mitotically derived gametes produce a completely homozygous sporophyte after selfing (Klekowski and Baker, 1966). Wilkie's work (1956), which demonstrated self incompatibility in *Pteridium aquilinum*, has stimulated the use of fern gametophytes in genetic research. Klekowski and his associates (1968, 1969) used genetic and morphological criteria to characterize pteridophyte gametophytes in terms of their probability for intragametophytic (functionally bisexual) and intergametophytic (functionally unisexual) mating.

This study was undertaken to determine whether the ontogeny of *Dryopteris ludoviciana* (Kunze) Small gametophytes differed from that reported for other dryopterid and thelypterid gametophytes (Kny, 1895; Waldemann, 1928; Nayar and Chandra, 1963), and to determine sex expression under controlled conditions. The effect of population density on sex expression was tested by culturing single isolated gametophytes and gametophytes in moderate and dense populations. The ontogeny and sex expression of *Thelypteris dentata* (Forsk.) E. St. John was also observed for comparison.

### METHODS AND MATERIALS

*Dryopteris ludoviciana* occurs in cypress and gum swamps, as well as in lime sinks, from Florida west along the Gulf Coast to Louisiana and north as far as North Carolina (Brown and Correll,

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<sup>1</sup> This paper is a portion of a thesis completed by the senior author for the Master of Science Degree, I. S. U., 1969.

1942). A detailed record of its occurrence is not available, but it is less common than other *Dryopteris* species.

Two *Dryopteris ludoviciana* sporophytes were greenhouse-grown after their collection on November 20, 1966, in Alachua County, Florida, in the Devil's Millhopper, a lime sink 5-6 miles northwest of downtown Gainesville. A voucher specimen (*John T. Mickel 1730*) is in the Iowa State University Herbarium. The plants used in this study were diploid ( $2n = 82$ ) (A. R. Smith, pers. comm.).

Fertile pinnae were cut from vigorous fronds and rinsed in running distilled water to remove foreign spores. The pinnae were then sealed in small manila envelopes. After most of the sporangia had dehisced (8-12 days at 20°C) the spores were transferred to small corked vials and utilized within two months.

All spores were sown on a sterile mixture of loam and coarse sand (4:1). Varying numbers of spores were sown to produce different densities. Population density was determined by counting all gametophytes visible at 30X. The average number of gametophytes per cm<sup>2</sup> was then determined. Dense populations contained 10-21 gametophytes per cm<sup>2</sup> and moderate populations had 1-3 per cm<sup>2</sup>. Isolated spores were cultured in small petri dishes.

Culture vessels containing only sterile soil were opened for watering and observation with the same frequency as the experimental cultures. Fern gametophytes appearing in these cultures reflected the extent of contamination (about one per cent). Densely sown cultures of *Thelypteris dentata* were also maintained under the same conditions of culture as *Dryopteris ludoviciana* and later were surveyed for the occurrence of gametangia.

Conditions of culture, including the use of soil as a substrate (Atkinson and Stokey, 1964), were selected to approximate the habitat of naturally occurring fern gametophytes. The cultures were maintained in a Percival growth chamber. Diffuse illumination was provided by white fluorescent tubes and incandescent bulbs at an intensity of 350-400 ft-c. Temperature at the soil surface was maintained at 22°C during the 12 hr light and 12 hr dark periods.

Samples of 24 or more gametophytes were removed for observation of development and sex expression at 25, 35, 45, 58, 72, and 111 days after sowing. An effort was made to select gametophytes representative of all sizes and stages. The gametophytes were placed in a petri dish or on a glass slide containing 1.75% lactose solution for microscopic examination. This medium prevented plasmolysis for 8–12 hours and did not hinder the dehiscence of antheridia or the motility of sperms.

#### GAMETOPHYTE ONTOGENY AND GAMETANGIA

Spores of *Dryopteris ludoviciana* are bilateral with a prominent perispore. A spring-collected sample (April to June) of 162 spores ranged from 24–50  $\mu$  long by 16–33  $\mu$  wide. One hundred and eight fall-collected spores (October) ranged from 21–54  $\mu$  long by 16–37  $\mu$  wide. The spring-collected spores averaged  $36 \times 24 \mu$  and the fall-collected spores averaged  $36 \times 26 \mu$ . Spore size was considerably less uniform in the fall collection (*Fig. 1*), but the biological significance of this, if any, is not known. A bimodal distribution of spore size was not found.

Most spores germinated within 6–20 days, but some took as long as three months. The spore coat ruptured irregularly or along the ridge and remained attached to the basal cell (*Fig. 2*). The first rhizoid was delimited shortly after germination, and a second rhizoid sometimes formed before filamentous growth began (*Fig. 3*). Successive divisions then produced a 4–6-celled filament which was either stout (*Fig. 4*) or elongate (*Fig. 5*). Filamentous growth was prolonged if a spore germinated in a soil crevice, or was otherwise shaded (*Fig. 7*). These attenuated filaments eventually became planate.

Longitudinal or oblique divisions in the terminal cell of the filament initiated planate growth (*Fig. 6*). These divisions could occur when the filament was only two or three cells long (*Fig. 8*). Subsequent longitudinal divisions could occur in all but the basal cell of the filament, obscuring the earlier filamentous form (*Fig. 9*).

Continued divisions in two dimensions were most frequent distally, producing a triangular gametophyte of 10–15 cells with

marginal trichomes (*Figs. 11, 12*). This stage was occasionally irregular (*Fig. 10*), but these irregularities were obscured by subsequent symmetrical growth. The fastest growing gametophytes established an apical cell 12–16 days after germination (*Fig. 13*;

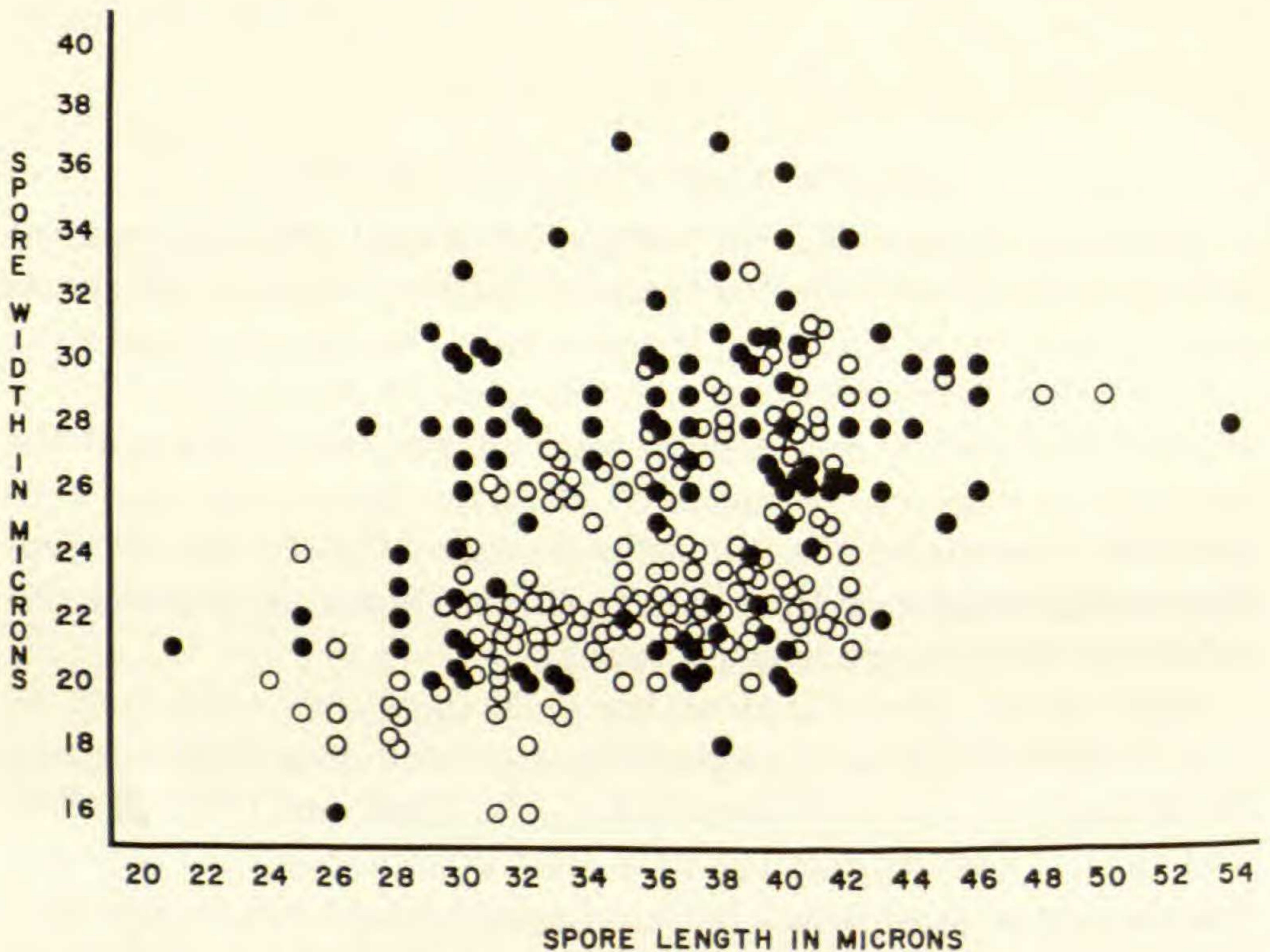


FIG. 1. SIZE DISTRIBUTION OF DRYOPTERIS LUDOVICIANA SPORES. ○ = ONE SPRING-COLLECTED SPORE; ● = ONE FALL-COLLECTED SPORE.

*Table I*). Slower growing gametophytes reached this stage within 16–30 days. The apical cell cut off derivatives laterally to produce the notch meristem 14–32 days after germination (*Figs. 14, 15*; *Table I*). Once this stage was reached, irregularities in growth were rare, and all gametophytes, except ameristic males which lack a notch meristem, became cordate (*Fig. 16*).

Trichomes were common on the dorsal surface, averaging one trichome per five prothallial cells. This ratio diminished to one

trichome per fifteen prothallial cells on the wings and to one per 20–30 prothallial cells on the ventral surface.

Gametophytes 20–45 days old and 0.5–0.7 mm wide initiated a cushion which was two or three cells thick at first and later four or five cells thick. Lateral prothallial cells (*Fig. 20*) became much larger than younger anterior cells (*Fig. 19*). Older gametophytes were 0.9–13.0 mm wide (*Figs. 17, 21*), depending on their pattern of sex expression.

TABLE I. INITIATION (IN DAYS AFTER GERMINATION) OF THE STAGES OF DEVELOPMENT OF *DRYOPTERIS LUDOVICIANA* GAMETOPHYTES.

<i>Developmental stage</i>	<i>Rapidly growing gametophyte</i>	<i>Slow growing gametophyte</i>
Filamentous	6	6
First longitudinal division	7	7
Early planate (9–15 cells)	8–11	11–29
Apical cell	12–16	17–30
Notch meristem	14–19	20–32
Cushion initiation	20–26	27–45

The antheridia are of the polypodioid type (Atkinson and Stokey, 1964), consisting of a single basal cell, a cylindrical middle cell which surrounds the central primary androgonial cell, and an undivided cap cell.

The archegonia are of the advanced type (Atkinson and Stokey, 1964) with four or five tiers of neck cells, each tier containing four cells. The central canal cell was binucleate and enlarged distally. The archegonium was slightly larger at its apex than at its base and was always inclined slightly toward the base of the gametophyte. Archegonia occurred ventrally on a broad area just behind the notch, and up to 80 occurred on gametophytes 8–13 mm wide.

#### SEX EXPRESSION IN POPULATIONS

*Dryopteris ludoviciana* has a normal sexual alternation of generations. The gametophytes observed in isolated, moderate,

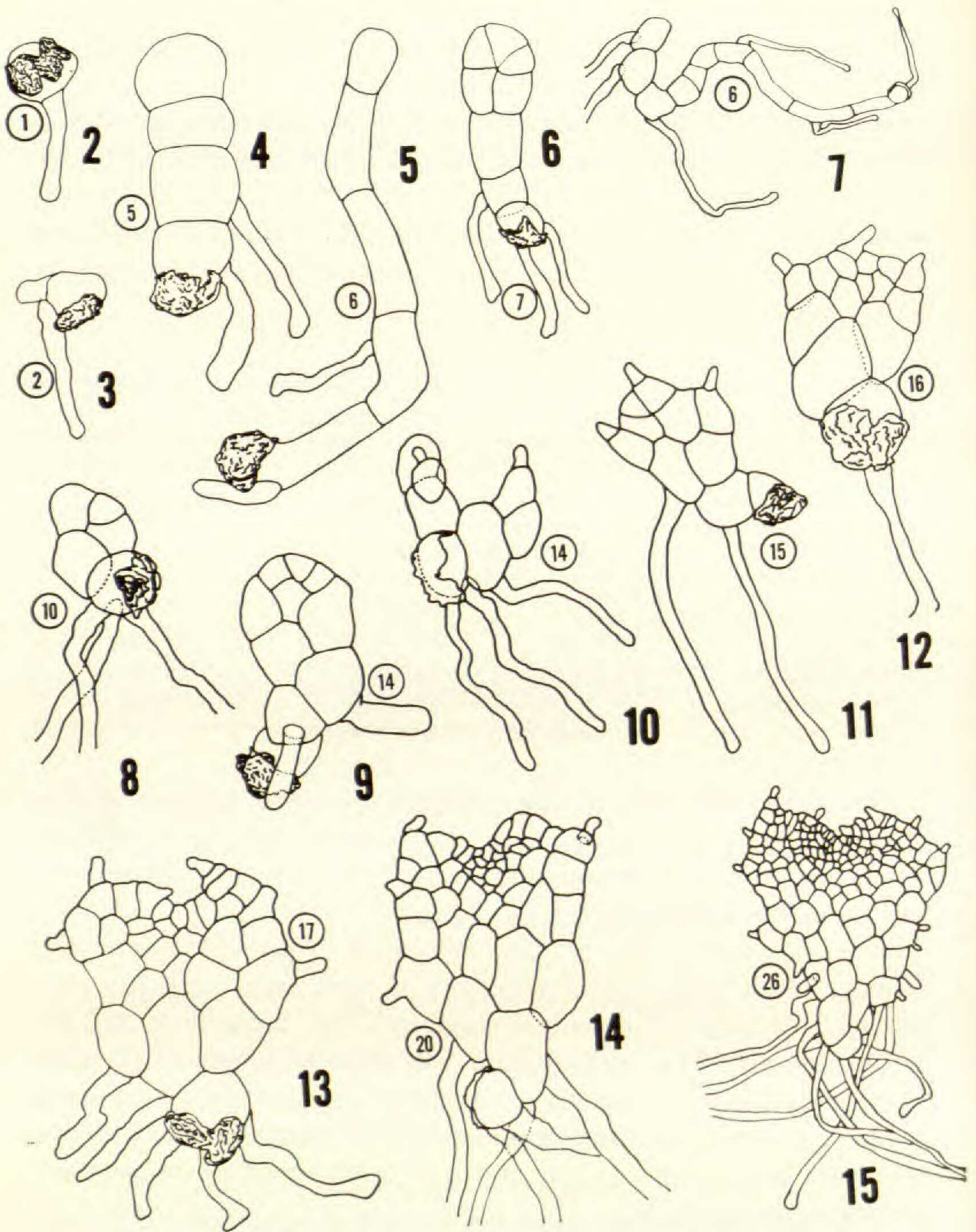


PLATE 5. EARLY ONTOGENY OF *D. LUDOVICIANA* GAMETOPHYTES. THE NUMBERS IN CIRCLES INDICATE DAYS AFTER GERMINATION. THE MOST COMMON SEQUENCE OF DEVELOPMENT IS FIGS. 2, 4, 6, 11-15. ONE CM = 100  $\mu$  FOR FIGS. 7, 15; 1.5 CM = 100  $\mu$  FOR ALL OTHER FIGURES.

and dense cultures expressed one of five possible patterns of sexuality, or lacked gametangia. The five sex expression patterns were based on the presence of one or both types of gametangia, and, where both kinds of gametangia occurred, the sequence of their appearance. The presence or absence of a notch meristem was also recorded for all gametophytes.

Rapidly growing gametophytes (*Table I*) usually became *unisexual females* and were the first plants in all populations to become sexual (*Fig. 18*). Archegonial initials were found on 24-day-old gametophytes as small as 0.9 mm wide. These gametophytes continued to grow and produce additional archegonia, attaining an average width of 4.6 mm 45 days after germination (*Fig. 21*). These gametophytes remained unisexual after 105 days and became 8–13 mm wide (*Fig. 17*). Older gametophytes occasionally produced basal outgrowths which became antheridial.

*Protandrous bisexual* gametophytes are typical of most homosporous leptosporangiate ferns (Atkinson and Stokey, 1964), but were rare in *D. ludoviciana* populations studied here. These gametophytes first produced antheridia among the rhizoids and on the basal half of the cushion. Six to 20 days later archegonia formed below the notch meristem. Antheridia numbered fewer than 80 on protandrous bisexual gametophytes. Twenty of the 527 gametophytes surveyed were protandrous bisexual.

In *synchronous bisexual* gametophytes archegonia occurred in the typical position below the notch meristem and antheridia were restricted to the distal half of the cushion adjacent to and among the archegonia. Maturation of both kinds of gametangia was synchronous, so that the periods of antheridial dehiscence and archegonial receptivity overlapped. These gametophytes first became sexual 30–40 days after germination and continued to produce both kinds of gametangia. After 45 days a gametophyte bore an average of ten archegonia and 22 antheridia.

*Ameristic male* gametophytes developed antheridia precociously but failed to develop an organizing notch meristem. They became sexual shortly after germination, producing antheridia on almost every cell, and occasionally two per cell. Ameristic males devel-

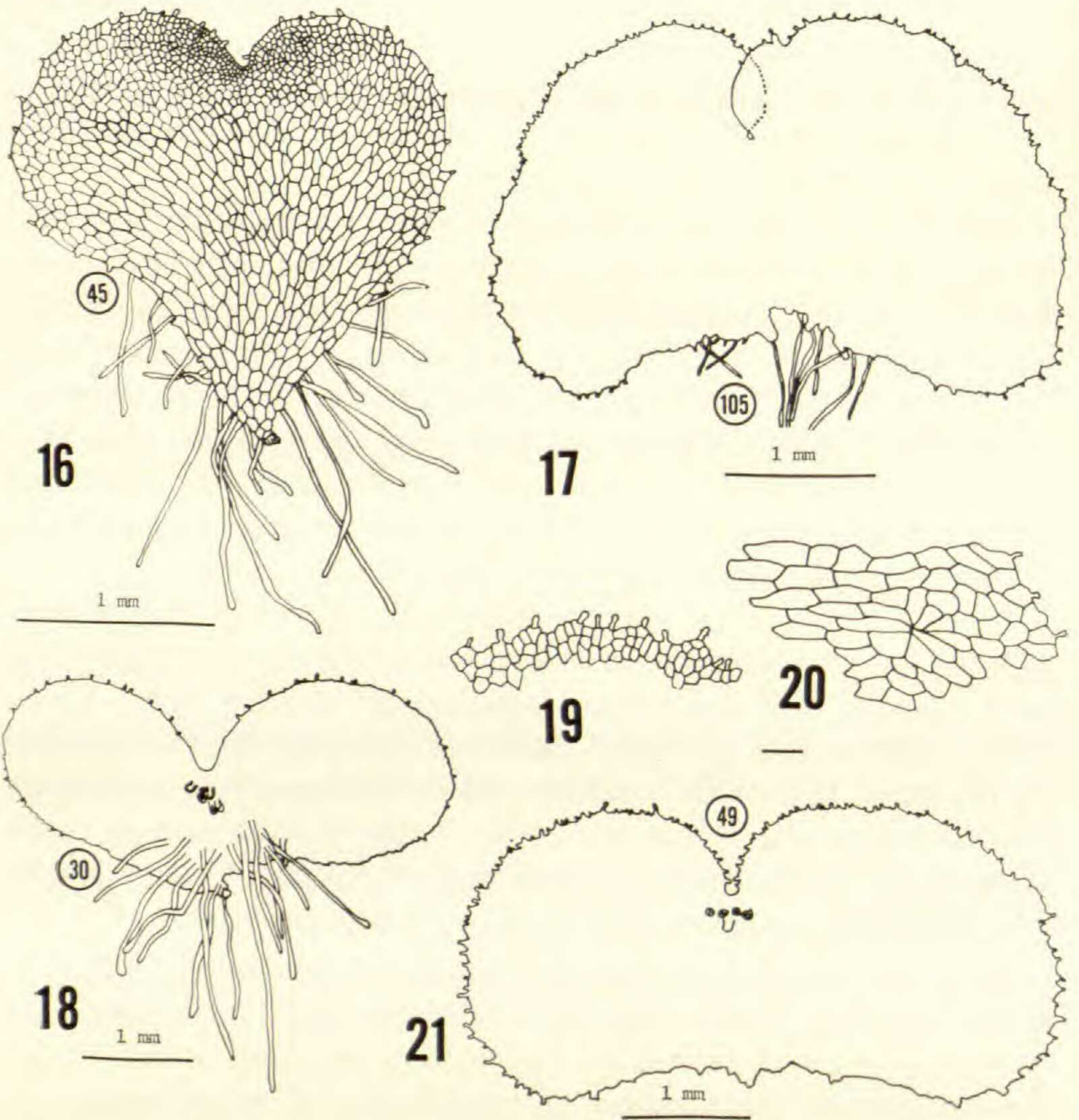


PLATE 6. LATE ONTOGENY OF *D. LUDOVICIANA* GAMETOPHYTES. THE NUMBERS IN CIRCLES INDICATE DAYS AFTER GERMINATION. FIG. 16. SLOW GROWING GAMETOPHYTE WITH CUSHION BUT WITHOUT GAMETANGIA. FIG. 17. UNISEXUAL FEMALE GAMETOPHYTE WITH OVERLAPPING WINGS ANTERIOR TO NOTCH; ARCHEGONIA NOT ILLUSTRATED. FIG. 18. RAPIDLY GROWING GAMETOPHYTE WITH ARCHEGONIA. FIG. 19. CELLS FROM ANTERIOR MARGIN OF WING; CF. FIG. 20. FIG. 20. CELLS FROM LATERAL AREA OF FIG. 21. FIG. 21. RAPIDLY GROWING UNISEXUAL FEMALE GAMETOPHYTE.



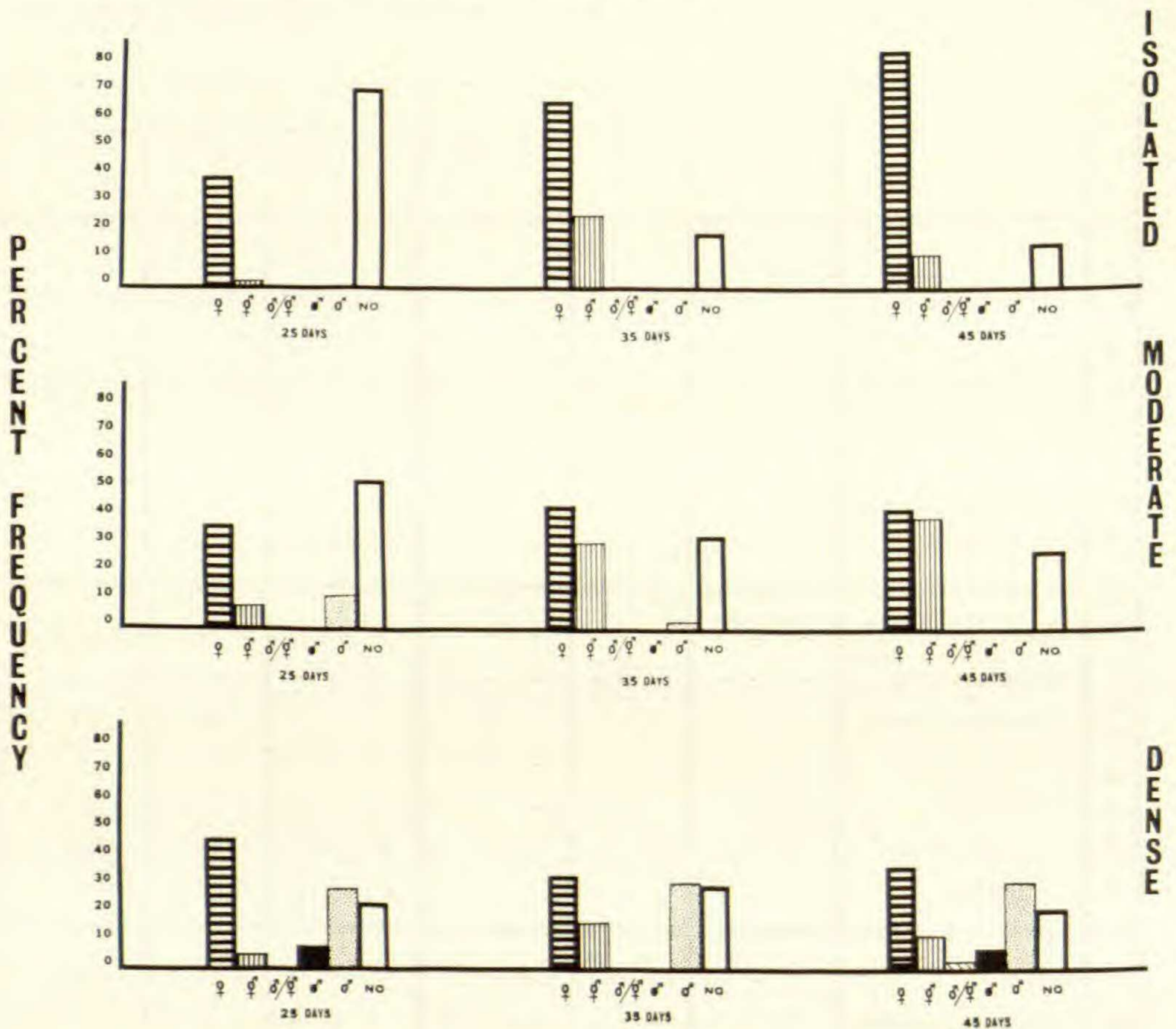
oped only from spores which either remained within sporangia, were next to sporangial fragments, or were in contact with the rhizoids of older gametophytes. Their form varied from filamentous to spathulate.

	SEXUALITY	SPRING						FALL											
		Per cent of total			Sample size			Average size mm			Per cent of total			Sample size			Average size mm		
		25	35	45	25	35	45	25	35	45	25	35	45	25	35	45	25	35	45
<b>I S O L A T E D</b>	FEMALE	28	-	75	34	-	6	1.9	-	2.6	43	60	80	13	18	20	1.6	3.3	6.1
	PROTANDROUS BISEXUAL	0	-	0	0	-	0	-	-	-	0	0	0	0	0	0	-	-	-
	SYNCHRONOUS BISEXUAL	1	-	12	2	-	1	2.1	-	3.6	0	23	8	0	7	2	-	3.9	6.3
	AMERISTIC MALE	0	-	0	0	-	0	-	-	-	0	0	0	0	0	0	-	-	-
	MERISTIC MALE	0	-	0	0	-	0	-	-	-	0	0	0	0	0	0	-	-	-
	GAMETANGIA ABSENT	71	-	13	90	-	1	0.4	-	4.0	57	17	12	17	5	3	0.4	0.5	0.8
	TOTALS	100	-	100	126	-	8	-	-	-	100	100	100	30	30	25	-	-	-
<b>M O D E R A T E</b>	FEMALE	-	-	-	-	-	-	-	-	-	34	40	39	10	14	11	1.5	2.9	3.7
	PROTANDROUS BISEXUAL	-	-	-	-	-	-	-	-	-	0	0	-	0	-	-	-	-	-
	SYNCHRONOUS BISEXUAL	-	-	-	-	-	-	-	-	-	7	28	36	2	10	10	1.8	2.5	3.1
	AMERISTIC MALE	-	-	-	-	-	-	-	-	-	0	0	0	0	-	0	-	-	-
	MERISTIC MALE	-	-	-	-	-	-	-	-	-	11	2	0	3	1	0	0.6	1.4	-
	GAMETANGIA ABSENT	-	-	-	-	-	-	-	-	-	48	30	25	15	11	7	0.5	0.3	0.9
	TOTALS	-	-	-	-	-	-	-	-	-	100	100	100	30	36	28	-	-	-
<b>D E N S E</b>	FEMALE	43	-	30	21	-	22	5.3	-	3.6	40	30	32	29	27	26	2.4	3.0	3.5
	PROTANDROUS BISEXUAL	0	-	2	0	-	2	-	-	3.5	0	0	1	0	0	1	-	-	5.0
	SYNCHRONOUS BISEXUAL	0	-	0	0	-	0	-	-	-	8	15	22	6	13	18	2.7	3.1	3.9
	AMERISTIC MALE	14	-	12	7	-	9	0.8	-	0.6	0	0	0	0	0	0	-	-	-
	MERISTIC MALE	33	-	20	16	-	14	1.7	-	1.5	20	28	36	14	25	29	0.8	0.6	0.9
	GAMETANGIA ABSENT	10	-	36	5	-	26	2.0	-	2.5	32	27	9	23	24	7	0.6	0.6	0.5
	TOTALS	100	-	100	49	-	73	-	-	-	100	100	100	72	89	81	-	-	-

TABLE II. SEX EXPRESSION IN 25, 35, AND 45-DAY-OLD ISOLATED, MODERATE, AND DENSE DRYOPTERIS LUDOVICIANA CULTURES.

*Meristic males* produced 100–300 antheridia after they became cordate. The antheridia occurred on two-thirds of the wings, and

extended to within four cells of the notch meristem. Meristic males continued to produce antheridia after being transplanted back to soil, and were not bisexual after 105 days.



### SEX EXPRESSION

FIG. 22. AVERAGE OF SEX EXPRESSION FOR SPRING AND FALL CULTURES COMBINED. THE SYMBOLS REPRESENT UNISEXUAL FEMALES, SYNCHRONOUS BISEXUAL GAMETOPHYTES, PROTANDROUS BISEXUAL GAMETOPHYTES, AMERISTIC MALES, MERISTIC MALES, AND GAMETOPHYTES WITHOUT GAMETANGIA.

The occurrence of meristic males, ameristic males, and protandrous bisexual gametophytes, and the frequency of all five sex expression types, varied with population density (*Table II*). Neither type of male occurred in isolated cultures, whereas moderate cultures 45 days and younger contained two to ten per

cent meristic males. Dense cultures had both types of unisexual male gametophytes at a 28–33 percent level.

Unisexual female and synchronous bisexual gametophytes occurred in all 25-day-old cultures (*Table II; Fig. 22*). Most of the unisexual females bore receptive archegonia at this time; most gametangia on synchronous bisexual gametophytes were immature.

Sexuality was expressed rapidly in dense cultures, as only 10–23 per cent of the gametophytes in 25-day cultures were without sex organs. Forty-eight per cent of the gametophytes in 25-day moderate cultures were asexual, whereas isolated cultures were slowest, with 57–71 per cent remaining asexual at 25 days. The proportion of gametophytes without sex organs decreased with time in all cultures from an average of 19 per cent at 45 days (*Table II; Fig. 22*) to zero at 105 days.

Protandrous bisexual gametophytes were first found in dense cultures at 45 days and they remained at a two to four per cent level during 105 days of culture. The proportions of other sex expression classes in dense cultures remained approximately uniform through time, except for the absence of ameristic males at 35 days (*Table II; Fig. 22*).

The proportion of synchronous bisexual gametophytes increased with time in moderate cultures, but the proportion of unisexual females remained uniform. In 58 and 72-day-old moderate cultures protandrous bisexual gametophytes occurred at a 10–15 per cent level and meristic males increased to 31–40 per cent.

The proportion of unisexual females in isolated cultures increased from 35 per cent at 25 days to 77 per cent at 45 days, while the proportion of unisexual females in moderate and dense cultures varied little from the initial percentage.

The *Thelypteris dentata* gametophytes which were cultured under the same conditions as *Dryopteris ludoviciana* expressed the protandrous bisexual pattern in all observed cases.

#### DISCUSSION

The ontogeny of *Dryopteris ludoviciana* as described here does not differ significantly from the typical cordate pattern of the

Polypodiaceae *sensu lato*. The ontogeny of *D. ludoviciana* does not distinguish it from either *Aspidium spinulosum* Swartz [*Dryopteris spinulosa* (Muell.) Watt.] or *A. thelypteris* Swartz [*Thelypteris palustris* Schott] as reported by Waldemann (1928). Kny's (1895) illustrations of the development of *Dryopteris filix-mas* gametophytes fail to distinguish it from *D. spinulosa*, *D. ludoviciana*, and *Thelypteris palustris*. It is likely that both *Dryopteris* and *Thelypteris* gametophytes have evolved to the same specialized level, and that gametophyte morphology is of little value in distinguishing between dryopterid and thelypterid ferns.

The five sex expression types found in *Dryopteris ludoviciana* populations allow intragametophytic mating and two levels of intergametophytic mating. Intragametophytic fertilization eliminates heterozygosity. Intergametophytic fertilization between gametophytes from the same sporophyte is equivalent to self-pollination and reduces heterozygosity. Intergametophytic fertilization between gametophytes derived from different sporophytes is equivalent to cross-pollination and increases heterozygosity (Klekowski, 1969).

The first sexual gametophytes observed in all populations were unisexual females, and the potential for intergametophytic fertilization was initially great. Intragametophytic fertilization became possible five to fifteen days later when synchronous bisexual gametophytes matured.

Although the pattern of spore distribution for naturally occurring *D. ludoviciana* plants is not known, it is likely that a greater number of spores will fall near the parent sporophyte and that spore density will decrease with distance from the parent plant.

Fertilization is most likely in dense populations because all five sex expression types occur and gametophytes often overlap. Fertilization is less likely in moderate populations and is least likely in isolation.

Dense populations from a single sporophyte have a high likelihood of intergametophytic gamete exchange which reduces heterozygosity, but are unlikely to effect exchange with gametophytes derived from different parent sporophytes. Synchronous bisexual

gametophytes would maintain a low level of intragametophytic fertilization in dense populations (*Table II; Fig. 22*). Moderate populations have the highest level of intragametophytic fertilization because of the high proportion of synchronous bisexual gametophytes. The potential for intergametophytic fertilization is lower than that of dense populations because fewer gametophytes overlap. Decreased population density would, however, increase the likelihood of establishment of a foreign spore. This would increase the possibility of intergametophytic fertilization at a level which increases heterozygosity.

Isolated bisexual gametophytes could establish sporophytes by selfing. Isolated unisexual female gametophytes would fail to establish a sporophyte, except where spore distribution patterns of different *D. ludoviciana* plants overlapped. The resulting sporophyte would greatly increase heterozygosity in the gene pool.

Variation of sex expression types found in different population densities implies the existence of a population factor which influences sex expression. The occurrence of unisexual males in dense and moderate cultures, but not in isolation, indicates that this factor influences maleness. Döpp (1950), who discovered an antheridium-inducing substance in *Pteridium aquilinum*, found that the *Pteridium* factor induced precocious antheridium formation in *Dryopteris filix-mas*, and an aqueous extract from *D. filix-mas* gametophytes induced antheridia on *P. aquilinum*.

Näf (1958, 1961) determined the mode of action of the *Pteridium* factor in *P. aquilinum* populations; his findings parallel certain aspects of the *Dryopteris ludoviciana* system. He found that unisexual females, ameristic males, and protandrous bisexual gametophytes occurred in *P. aquilinum* populations. Isolated gametophytes became unisexual females only, while all gametophytes became ameristic males if cultivated under conditions of slow growth with added *Pteridium* factor. Susceptibility to the induction of antheridia ceased before archegonia were formed, and the fastest growing gametophytes in the population probably attained the insensitive phase before the concentration of the factor was sufficient to induce antheridia.

The fastest growing *Dryopteris ludoviciana* gametophytes also become unisexual females, and maleness does increase with population density. The occurrence of bisexual gametophytes in isolation, however, cannot be the result of an antheridium-inducing substance secreted by other gametophytes. The antheridium-inducing factor also fails to explain the typical bisexual pattern of the majority of leptosporangiate ferns.

It is possible that the typical bisexual condition of most fern gametophytes is the ancestral condition, and that the *P. aquilinum* system is derived. In this latter case all spores are potential unisexual female gametophytes, and other sex expression types are determined by the interaction of an antheridium-inducing substance and variation in growth rate. If this is so, then the *D. ludoviciana* system is an intermediate one which shares the advantages of both the ancestral and advanced conditions.

At its present level of evolution the *D. ludoviciana* system has three favorable adaptations which increase its evolutionary potential. Sporophyte production is guaranteed in all population densities by a persistent level of the ancestral bisexual condition. Where a sporophyte population is already established, the level of heterozygosity is maintained by two levels of intergametophytic fertilization, so that the ability of the population to adapt to a changing environment is not lessened. The potential to increase heterozygosity is highest at the limit of the range so that range extension is possible by gamete exchange between neighboring *D. ludoviciana* populations. Finally, unlike *P. aquilinum*, some isolated spores can produce bisexual gametophytes which may self to form a sporophyte. This would allow range extension of the species throughout a habitat and disjunct range extension when scattered spores occur in a suitable habitat far removed from that of the parent sporophyte.

The role of the gametophyte in the natural distribution of ferns has not been assessed. Fern spores do act as long distance propagules, but the establishment of a gametophyte does not guarantee sporophyte occurrence (Farrar, 1967). Both gametophyte and sporophyte are subjected to selection in range exten-

sion of the species. Gametophyte adaptations, which would seem to encourage colonization, may be ineffective if the sporophyte is limiting. The relative effectiveness of gametophytes and sporophytes in colonization, the frequency of gametophyte establishment, and the occurrence of sex expression types must now be determined in the field.

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